Wednesday, May 11, 2016 (Margaret Lie)

Objectives: To amplify copies of the arabinose-inducible iRFP 670 and 713 plasmid.

1. Transformed pBAD-His-B-iRFP670 and pBAD-His-B-iRFP713 into E. coli XL1 Blue electrocompetent.
    a. See Electroporation protocol.
2. Plated on Luria Bertani plates with 100 µg/mL ampicillin (50 µL straight) and 50 µg/mL ampicillin (100 µL of 1:100 and 1:1000 dilutions).
3. Incubated 16 h at 37C.

Objectives: To amplify copies of the arbinose-inducible violacein plasmid (Vio ABDE+ribozyme).

1. Transformed BBa_K598019 (violacein arabinose 2) into E. coli XL1 Blue electrocompetent.
    a. See Electroporation protocol.
2. Plated on Luria Bertani plates with 34 µg/mL chloamphenicol.
3. Incubated 16-18 h at 37C.

Thursday, May 12, 2016 (Margaret Lie)

Objectives: To count colonies

1. Parafilmed plates and stored at 4C on Josh's shelf.
2. For iRFP 670, lawns for 50 µL and 1:100. 1000s of colonies for 1:1000.
3. For iRFP 713, lawns for 50 µL and 1:100. 1000s of colonies for 1:1000.

Wednesday, May 18, 2016 (Jessica Lee)

Objectives: Set up colony PCR.

1. Grew 3 mL cultures from 3 colonies.
    a. See colony PCR protocol
    b. 3 mL = Luria Bertani broth + 100 µg/mL ampicillin for iRFP
    c. 3 mL = Luria Bertani broth + 34 µg/mL chloramphenicol for Vio ABDE+ribozyme
2. Incubated culture tubes at 37C, shaking (250 rpm) for 16-18 h.

Thursday, May 19, 2016 (Jessica Lee)

Objectives: Obtained amplified plasmid.

1. Followed QIAprep Spin MIniprep Kit protocol (Hilden, Germany).
2. Used Nanodrop to obtain concentrations.
3. Stored violacein DNA (labelled V#1 and V#2), iRFP 670#1 and #2, and iRFP 713 #1 and #2 in purple box in Ilenne's rack.

Friday, May 20, 2016 (Jessica Lee)

Objectives: Transformation of AraC in *E. coli*

1. Added 79 µL H_2O, 1 µL V#1 DNA, 20 µL 5X KCM, and 100 µL *E. coli* provided by David Zong in the Matthew Bennett Lab.
2. Placed tube on ice for 20 minutes.
3. Placed tube at 42°C for 1 minute 30 seconds.
4. Placed tubes immediately back on ice and added 1 mL LB.
5. Incubated tube 1 h at 37°C.
6. Plated 1:1, 1:100, and 1:1000 dilution of cultures onto LB + 34 µg/mL chloramphenicol plates.

Monday, May 23, 2016 (Jessica Lee)

Objectives: Testing arabinose induction.

1. Grew 5 mL culture in LB with colony from 1:1000 violacein plate.
2. Incubated at 37°C, 250 rpm for 18 h.
3. MW arabinose = 150.13 g
    a. 0.002 L x 1 mol/L x 150.13 g/mol = 0.3 g arabinose
4. Added 5 µL 1 M arabinose to 5 mL violacein culture. → Final concentration= 1 mM.
5. Added 5 µL 1 M arabinose to 5 mL negative control.

Hypothesis: Culture #1 = purple. Culture #2 = NOT purple.

Observations: Colonies more brown than purple.

Wednesday, May 25, 2016 (Jessica Lee)

Objectives: Analyzed product by fluorescence.

1. Transferred dilutions of cultures in 96 well plate.
2. Used Tecan m1000 microplate reader to analyze fluorescence.

Observations: Fluorescent signals not ideal possible because VioC enzyme missing.